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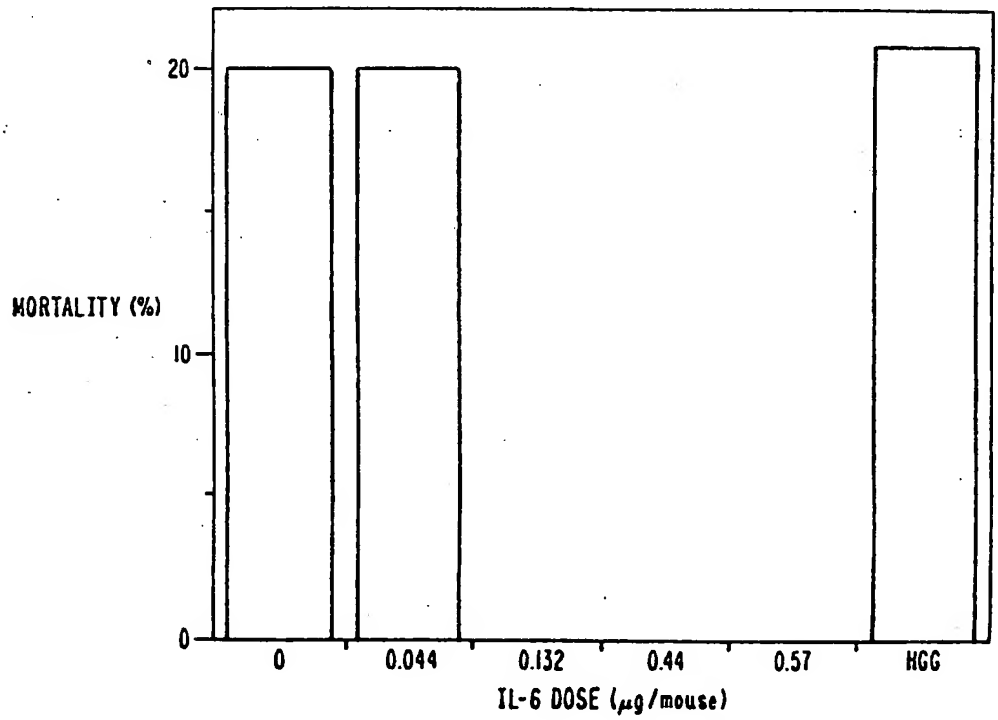
(54) Title: USE OF THE COMBINATION OF ANTI-TUMOR NECROSIS FACTOR PLUS INTERLEUKIN-6 TO TREAT SEPTIC SHOCK



(57) Abstract

Methods and compositions are provided for treating or preventing septic shock in a mammal. The methods comprise administering to a mammal afflicted with or at high risk for developing septic shock and effective amount of a combination of an anti-TNF antibody and IL-6.

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USE OF THE COMBINATION OF ANTI-TUMOR NECROSIS FACTOR PLUS INTERLEUKIN-6 TO TREAT SEPTIC SHOCK

BACKGROUND OF THE INVENTION

Septic shock is an often fatal condition usually resulting from gram-negative bacteremia. Despite the use of potent antibiotics and intensive care, the mortality with sepsis and gram-negative bacteremia remains high (20-60% depending on the specific population) [Ziegler *et al.*, New Eng. J. Med. *324*:429 (1991); Bone *et al.*, New Eng. J. Med. *317*:653 (1987); and Kreger *et al.*, Am. J. Med *68*:344 (1980)]. Approximately 100,000-300,000 cases of gram-negative bacteremia caused sepsis are reported per year, with the resulting deaths estimated at 30,000 to 100,000 [Wolff, New Eng. J. Med. *324*:486 (1991)]. Sepsis requires prompt treatment, since the patient's condition often deteriorates rapidly. It is a leading cause of morbidity and mortality among hospitalized patients. The symptoms of septic shock include fever or hypothermia, tachycardia, tachypnea, hypotension, peripheral hypoperfusion or systemic toxicity. [Ziegler *et al.*, *supra*].

Tumor necrosis factor (TNF), a pro-inflammatory cytokine, is thought to play a major role in the pathogenesis of septic shock [Franks *et al.*, Infec. Immunol. *59*:2609 (1991)]. Administration of neutralizing antibodies to murine or human TNF has been shown to protect mice, rabbits, and primates against death from the experimentally-induced manifestations of septic shock. [Dinarello *et al.*, J. Infec. Dis. *163*:1177 (1991); Tracey *et al.*, Nature *330*:66 (1987); Beutler *et al.*, Science *229*:869 (1985); Mathison *et al.*, J. Clin. Invest. *81*:1925 (1988)].

The role of Interleukin-6 (IL-6) in septic shock has not been as clearly defined. One investigator has reported a new link between TNF and IL-6; IL-6 can reduce the experimentally-induced release of TNF in the monocytoid cell line U937, cultured human peripheral blood monocytes, and intact mice [Aderka *et al.*, J. Immunol. *143*:3517 (1989)]. Other investigators have demonstrated that TNF is a potent inducer of IL-6 in cultured fibroblasts [Kohase *et al.*, Cell *45*:659 (1986)], in various tumor cell lines [Defilippo *et al.*, Proc. Natl. Acad. Sci. (USA) *84*:4557 (1987)], and also in man [Jablons *et al.*, J. Immunol. *142*:1542 (1989)].

The existence of a reciprocal stimulatory/inhibitory interaction between TNF and IL-6 suggests a complex relationship of considerable potential importance in the regulation of the many varied biologic actions of these two cytokines [Aderka *et al.*, *supra*]. The inhibitory effect of IL-6 on TNF production possibly reflects a predominantly anti-inflammatory function of IL-6 [Aderka *et al.*, *supra*]. IL-6 has also been shown to induce ACTH release and thereby induces cortisol synthesis, which further enforces the suggestion that IL-6 sometimes serves an anti-inflammatory function [Woloski *et al.*, Science *230*:1035 (1985)].

SUMMARY OF THE INVENTION

This invention provides a method for treating septic shock in a mammal comprising administering to a mammal afflicted with septic shock an effective amount of a combination of an anti-TNF antibody and IL-6. This invention also provides a method for preventing septic shock in a mammal which comprises administering to a mammal susceptible to or at high risk for developing septic shock, an effective amount of a combination of an anti-TNF antibody and

IL-6. A pharmaceutical composition comprising a combination of an anti-TNF antibody and IL-6, and a physiologically acceptable carrier, is also provided by this invention.

BRIEF DESCRIPTION OF THE FIGURES

This invention can be more readily understood by reference to the accompanying Figures, in which:

Fig. 1 is a graphical representation of the effects of various treatments administered to groups of 20 mice 18 hours prior to challenge with LPS-gal. Mortality in the groups of mice 24 hours after LPS-gal challenge is shown as a function of pre-treatment with control Dulbecco's phosphate buffered saline (DPBS) and monoclonal antibodies against IL-6 (20F-3) and IL-5 (TRFK-5).

Fig. 2 is a graphical representation of the effects of various treatments administered to groups of 20 mice 18 hours prior to challenge with LPS-gal. Mortality in the groups of mice 24 hours after LPS-gal challenge is shown, from left to right, as a function of pre-treatment with control Dulbecco's phosphate buffered saline (DPBS), 500 µg of hamster gamma globulin (HGG), and 50 µg and 100 µg of an anti-TNF antibody (TN3) (hamster origin).

Fig. 3 is a graphical representation of the effects of varying doses of anti-TNF antibody administered to groups of 20 mice 18 hours prior to challenge with LPS-gal. Mortality in the groups of mice 24 hours after challenge is shown as a function of pre-treatment antibody dose.

Fig. 4 is a graphical representation of the effects of various treatments administered to groups of 20 mice prior to challenge with LPS-gal. Mortality in the groups of mice 24 hours after LPS-gal challenge is shown as a function of pre-

treatment with 25 μg/mouse anti-TNF antibody (TN3) with or without 1 mg/mouse anti-IL-6 antibody (20F-3, or with Dulbecco's phosphate buffered saline (DPBS) or 1 mg/mouse hamster gamma globulin (HGG). The results from two experiments are shown using TN3 with or without 20F-3; the DPBS and HGG values shown are the averages from the two experiments. For the combination treatments, $p<0.05$ as determined by the Student's *t*-test.

Fig. 5 is a graphical representation of the effects of varying doses of recombinant IL-6 or 0.57 μg/mouse control hamster gamma globulin (HGG) administered to groups of 20 mice 1 hour prior to challenge with LPS-gal. The mice had also been treated with 25 μg/mouse anti-TNF antibody prior to LPS-gal challenge. The mortality in the groups of mice 24 hours after challenge is shown as a function of IL-6 dose.

Fig. 6 is a graphical representation of the effects of varying doses of recombinant IL-6 administered to groups of 20 mice 1 hour prior to challenge with LPS-gal. Mortality in the groups of mice 24 hours after challenge is shown as a function of IL-6 dose. The two bars for each IL-6 dose represent the results of two separate experiments. For both IL-6 doses, $p > 0.07$ as determined by the Student's *t*-test.

DESCRIPTION OF THE INVENTION

All references cited herein are hereby incorporated in their entirety by reference.

The term "septic shock" as used herein is defined as a state of morbidity manifesting one or more of the following symptoms: fever or hypothermia [temperature above 38.7°C (101° F) or below 35.6° C (96° F)]; tachycardia (heart rate above 90 beats per minute in the absence of a beta-blockade), tachypnea (respiratory rate above 20 breaths

per minute or the requirement of mechanical ventilation); and either hypotension (systolic blood pressure below 90 mm Hg or a sustained drop in systolic pressure above 40 mm Hg in the presence of adequate fluid challenge and the absence of anti-hypertensive agents) or two of the following six signs of systemic toxicity or peripheral hypoperfusion: unexplained metabolic acidosis (blood pH below 7.3, base deficit of greater than 5 mmol per liter, or an elevated plasma lactate level); arterial hypoxemia (partial pressure of oxygen below 75 mm Hg or ratio of the partial pressure of oxygen to the fraction of inspired oxygen less than 250); acute renal failure (urinary output of less than 0.5 ml per kilogram of body weight per hour); elevated prothrombin or partial thromboplastin time or reduction of the platelet count to less than half the baseline value or less that 100,000 platelets per cubic milliliter; sudden decrease in mental acuity; and cardiac index of more than 4 liters per minute per square meter of body-surface area with systemic vascular resistance of less than 800 dyn · sec · cm^{-5}; and serum elevation of TNF [Ziegler *et al.*, *supra*].

The symptoms listed above are illustrative of specific selection criteria to be used in determining candidates for the proposed method of treatment. States of morbidity which can cause the foregoing symptoms include but are not limited to: acute gram-negative bacteria infections, endotoxemia, purpura fulminans, severe psoriasis, acute rheumatoid arthritis, burns, organ transplant rejection, and physical traumas, such as abdominal wounds. Candidates for abdominal surgery (especially bowel surgery) are at high risk for developing septic shock [Debets *et al.*, Crit. Care Med. *17*(6):489 (1989)] and could benefit from prophylactic administration of the combination of an anti-TNF antibody and IL-6.

The effectiveness of treatment can be assessed by monitoring the above mentioned manifestations of septic shock.

Anti-TNF antibodies are available commercially, e.g., Boehringer Mannheim Biochemicals, Indianapolis, IN. IL-6 is commercially available from Genzyme Corporation, Cambridge, MA. Both can also be prepared by known methods using natural sources or recombinant DNA methodologies [Sheehan *et al.*, J. Immunol. *142*:884 (1989); Starnes *et al.*, J. Immunol. *145*:4185 (1990)].

These materials are generally supplied in lyophilized form and can be reconstituted just prior to use in a pharmaceutically acceptable carrier such as phosphate buffered saline or any of the other well known carriers. The pharmaceutical compositions of the invention can be injected directly into the bloodstream intravenously or via an I.V. drip solution such as Ringer's lactate. Parenteral preparations that can be used include sterile solutions or suspensions. These preparations can be prepared with conventional pharmaceutically acceptable excipients and additives such as stabilizers and carriers. The solutions to be administered may be reconstituted lyophilized powders which may additionally contain, e.g., preservatives, buffers and dispersants. Preferably, the compositions are administered by intravenous injection.

Kits are also provided by this invention comprising anti-TNF antibodies and IL-6 in physiologically acceptable carriers, in separate containers.

Of course, all of the monoclonal antibodies can be modified by standard recombinant DNA techniques. Such techniques include but are not limited to the production of antibody variants that combine the rodent variable or hypervariable regions with the human constant or constant

and variable framework regions [Rudikoff *et al.*, Proc. Natl. Acad. Sci. USA *79*:979 (1982), Morrison and Oi, Adv. Immunol. *44*:65 (1989), Queen *et al.*, Proc. Natl. Acad. Sci. USA *86*:10029 (1989)]. Humanized antibodies can be generated in which the antigen binding complementarity determining regions (CDRs) from the parent rodent monoclonal antibody are grafted into a human antibody framework. These antibodies are less immunogenic [Queen *et al.*, *supra*]. Humanized rodent antibodies also demonstrate a longer half-life in humans *in vivo* than their unmodified rodent counterparts [LoBuglio *et al.*, Proc. Natl. Acad. Sci. USA *86*:4220 (1989)].

An alternative approach to the production of monoclonal antibodies entails the cloning of the V-region genes from B-cells using the polymerase chain reaction technique. Antibody derivatives are then expressed in a microbial system (e.g. *E. coli*) and screened for antigen binding ability [Winter and Milstein, Nature *349*:293 (1991); Mullinax *et al.*, Proc. Natl. Acad. Sci. USA *87*:8095 (1990)]. Heavy and light chain libraries can be prepared in phage lambda and used to generate a large array of random heavy plus light chain pairs expressed in bacteria [Mullinax *et al.*, *supra*, and Waldmann, Science *252*:1657 (1991)].

IL-6 can be made if desired using standard recombinant DNA methods. For example, oligonucleotide probe mixtures based on known IL-6 nucleotide sequences can be used to identify DNA encoding IL-6 in genomic or cDNA libraries prepared by standard methods. DNA thus identified can be excised from the library by restriction endonuclease cleavage or prepared using appropriate primers and the polymerase chain reaction (PCR) method [Saiki *et al.*, Science *239*:487 (1988)], sequenced and expressed in a eukaryotic expression system or (following intron deletion by standard methods if necessary) in a prokaryotic or eukaryotic

expression system. Of course, both cDNA and genomic DNA libraries can be screened by the application of standard expression cloning methods, instead of by the use of oligonucleotide probes or PCR. IL-6 thus produced is detected through the use of known immunochemical or bioassay methods.

The anti-TNF and the IL-6 used will preferably be those of the mammalian species being treated (e.g., anti-human TNF and human recombinant IL-6 are preferred for treating human beings). It is also preferred that glycosylated IL-6 be used (e.g., recombinant IL-6 produced in a eukaryotic expression system).

In accordance with the present invention, mammals that are in need of treatment for septic shock as defined above are administered an effective amount of anti-TNF antibodies in combination with IL-6 to accomplish the above-described results. A dose of from about 0.5 μg to about 250.0 μg anti-TNF antibodies per kilogram of body weight and about 1.0 μg to about 3.0 mg IL-6 per kilogram of body weight is preferably administered. More preferably, mammals are administered a dose from about 1.0 μg to about 3.0 μg anti-TNF antibody per kilogram of body weight and from about 5.0 μg to about 30.0 μg IL-6 per kilogram of body weight. In humans, administration of the anti-TNF antibody can be concomitant with or prior to administration of the IL-6. The precise amount of the combination of the anti-TNF antibody and the IL-6 to be administered would be determined by the attending clinicians, taking into account the etiology and severity of the disease, the patient's condition, sex, age, and other factors.

In the Example below, overnight treatment of mice with the antibodies and cytokines investigated was done in order to facilitate adequate circulating concentrations of these materials in the bloodstream at the time of LPS-gal administration, because intraperitoneal injection of these materials requires a longer diffusion period to enter the bloodstream than other routes of administration (such as intravenous injection). Intraperitoneal injection was selected in this model due to the difficulty of intravenous injection in the mouse. The preferred route of administration would normally be intravenous injection, where bioavailability of the circulating therapeutic agents would be as rapid as 10 minutes.

EXAMPLE

The present invention can be illustrated by the following, non-limiting Example.

Materials

Male C57BL/6J mice (5 weeks of age) were obtained from Jackson Laboratories, Bar Harbor, ME. Endotoxin-free phosphate buffered saline (PBS) was purchased from GIBCO, Grand Island, NY. LPS (*E. coli* 0111:B4) was purchased from List Biological Laboratories, Inc., Campbell, CA. D-galactosamine was obtained from Sigma Chemicals, St. Louis, MO. Purified Recombinant TNF is available from Genzyme, Cambridge, MA and IL-6 is available from Biosource, Camarillo, CA; both were used as standard proteins. Monoclonal rat anti-mouse IL-6 , 20F-3 was obtained from DNAX Research Institute of Cellular and Molecular Biology, Palo Alto, CA. Anti-mouse TNF (purified TN3-19.12 Ab) was obtained from Dr. Robert D. Schreiber, Washington University, St. Louis, MO. Purified hamster gamma globulin (HGG), a protein control, was purchased from Cappel, Durham, NC. The

purified rat anti-mouse IL-5 was obtained from DNAX Institute of Cellular and Molecular Biology, Palo Alto, CA.

Demonstration of Protective Effects

LPS, or endotoxin, a component of the outer membrane of gram-negative bacteria, is responsible for the toxic manifestations associated with septic shock. Administration of purified LPS is a well-established method for experimentally inducing septic shock in animal models [Calandra *et al.*, Diag. Microbiol. Infect. Dis. *13*:377]. D-galactosamine is a hepatotoxin shown to potentiate the lethal effects of endotoxin (LPS) up to 100,000 fold [Galanos *et al.*, Proc. Natl. Acad. Sci. USA *76*:5939 (1979)]. This model is predictive of clinical utility in mammals afflicted with septic shock [Franks *et al.*, *supra*].

LPS was dissolved in PBS at 1 mg/ml and frozen at -80°C until use. Prior to freezing it was sonicated for 5 minutes in a sonifying bath. It was re-sonicated for 5 minutes after thawing. Appropriate dilutions were made in PBS in polypropylene tubes. D-galactosamine was dissolved at 32 mg/ml in PBS and mixed with an equal volume of diluted, sonciated LPS. The LPS-galactosamine mixture was used immediately and fresh batches were made for each experiment. Each mouse received 0.5 ml of LPS-gal mixture intraperitoneally (i.p.) between 1 and 3 pm. Animals were scored for mortality 24 hours later. All therapeutic agents were administered at the times indicated.

Blood samples were collected from anesthetized mice in serum separator tubes and allowed to clot overnight at 4°C. Sera were removed after microcentrifugation for 5 minutes at 2080 x g. IL-6 and TNF concentrations were measured in sera obtained 90 minutes after LPS-gal administration. Cytokine-specific enzyme linked

immunosorbent assays (ELISA's) were performed essentially as described by Sheehan *et al.*, and Starnes *et al.*, *supra*.

Doses of LPS ranging from 6.25 to 200 ng/mouse were injected i.p. with D-glactosamine as described. As shown in Table I, a dose dependent relationship was observed up to 100 ng/mouse (90% mortality). At higher doses the relationship was not observed. 100 ng/ml was selected as the dose to be used in subsequent experiments because it was found to be the dose that would yield the highest mortality at the lowest dose. In parallel experiments where the D-galactosamine was not co-administered with the LPS, animals survived doses of 1.5 mg of LPS for greater than 72 hours.

To identify the role of IL-6 in this lethal septic shock model, mice were injected i.p. with 1 and 2 mg/mouse anti-IL-6 antibody (20F-3) 1 to 2 hours prior to LPS-gal treatment. [Starnes *et al.*, *supra*]. Equal amounts of the isotype control of anti-IL-5 antibody (TRFK-5) were used as control proteins. Table I shows that there was no effect on mortality with the anti-IL-6 antibody treatment.

TABLE I

EFFECTS OF LPS DOSE ON MORTALITY

LPS/mouse (ng)	Ab/mouse (mg)	Mortality %
0	0	0
6.13	0	57±8
12.5	0	57±10
25	0	64±20
50	0	78±14
100	0	90±13
150	0	70±4
200	0	70±8
100	1[a]	85±8
100	2[a]	95±5
100	1[b]	100±0

[a] Ab was anti-IL-6 antibody
[b] Control Ab was anti-IL-5 antibody

Because of the long circulating half-life of the isotype of anti-IL-6 antibody (10 days to 2 weeks) the antibody was given the night before LPS-gal administration, in case 1 hour was not long enough for all of the anti-IL-6 antibody to enter the circulation from the peritoneal cavity. Results are shown in Fig. 1. Even with overnight treatment, the anti-IL-6 antibody failed to confer any protection against mortality.

As shown in Fig. 2, mice given 50 and 100 µg i.p. of anti-TNF antibody were protected from death. In this representative experiment, no mice died with overnight treatment using 100 µg anti-TNF antibody, and only 10% died after receiving 50 µg of the antibody the night before LPS-gal

administration. All mice given only the vehicle control or HGG control antibody died.

Fig. 3 shows the average dose relationship of mortality vs. treatment with anti-TNF antibody. Twenty-five μg/mouse given i.p. the night before LPS-gal administration conferred about 70% protection from death in this model. Doses lower than 10 μg/mouse conferred very little protection.

Treatment with anti-IL-6 antibody potentiated mortality when TNF was partially neutralized, as shown in Fig. 4. Mice were treated simultaneously with 1 and 2 mg of anti-IL-6 antibody and 25 μg/mouse anti-TNF antibody 18 hours prior to LPS-gal administration. Surprisingly, anti-IL-6 antibody was found to enhance mortality significantly. In one experiment where anti-TNF antibody only resulted in 25% mortality, the same dose plus anti-IL-6 antibody resulted in 65% mortality. In another experiment where the anti-TNF resulted in 20% mortality, the anti-TNF antibody plus IL-6 combination resulted in 45% mortality. These data suggest a protective role of IL-6 in this lethal shock model.

Doses of recombinant IL-6 were determined as follows: mice injected with 100 ng of LPS-gal were bled 90 minutes later. Sera were collected and analyzed for TNF and IL-6 concentration at this time point because this is the time determined for peak concentrations of TNF. Table II illustrates the results allowing for maximum volume of 10 ml/mouse (circulating blood volume plus partitioning into tissue and iterstitial spaces). It was calculated that 100 ng of LPS-gal treatment resulted in 440 ng of IL-6 per mouse. Therefore, 440 ng was the selected dose, plus higher and lower doses in half-log increments to measure the effect of recombinant IL-6 when TNF was partially limited by anti-TNF treatment.

TABLE II

SERUM LEVELS OF IL-6 AND TNF AFTER LPS/GAL ADMINISTRATION

Time (hours)	TNF, (ng/ml)	IL-6, (ng/ml)
0	0	0
1.5	2±1	44±13

Based upon the foregoing results, doses of IL-6 for therapeutic administration were selected, with the results shown in Fig. 5.

Fig. 5 shows that treatment with recombinant mouse IL-6 protected against mortality when TNF was limited by prior administration of anti-TNF antibody. Recombinant IL-6 was given i.p. 1 hour prior to LPS-gal administration. Mice had been treated the night before with 25 µg/each of anti-TNF antibody. At doses of 132 to 570 ng/mouse, recombinant IL-6 conferred significant protection against mortality. Mortality was lowered from an average in these experiments from 20-0% ($p < 0.05$) at 132, 440, and 570 ng/mouse. At lower doses (44 ng and less) no effect was observed. These results demonstrate that the combination of anti-TNF antibody and recombinant IL-6 as a treatment for septic shock is effective in significantly reducing mortality.

In contrast to the results shown in Fig. 5, IL-6 administration 1 hour prior to LPS-gal challenge was substantially ineffective in reducing mortality when the mice did not receive prior treatment with anti-TNF antibody. This is shown in Fig. 6, where the effects observed with the administration of 0.44 or 0.57 µg/mouse IL-6 were similar to

the results produced by Dulbecco's phosphate buffered saline alone (no IL-6).

The effect of the anti-TNF antibody plus IL-6 is best illustrated by comparing Fig. 3 with Fig. 5. In Fig. 3, 25 μg/ml of the anti-TNF antibody conferred only 70% protection from death, where in Fig. 5 that same dose of 25 μg/ml plus IL-6 (at 132, 440, and 570 ng/ml) conferred 100% protection from death.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will become apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims.

WHAT IS CLAIMED IS:

1. A method for treating septic shock comprising administering to a mammal afflicted with septic shock an effective amount of a combination of anti-TNF antibody and IL-6.

2. A method for preventing septic shock comprising administering to a mammal susceptible to or at risk for developing septic shock an effective amount of a combination of an anti-TNF antibody and IL-6.

3. A method for the manufacture of a pharmaceutical composition for treating or preventing septic shock comprising admixing a combination of an anti-TNF antibody and IL-6 with a pharmaceutically acceptable carrier.

4. The method of any one of claims 1 to 3 in which the anti-TNF antibody is an anti-human-TNF antibody and the IL-6 is recombinant human IL-6.

5. A pharmaceutical composition for treating or preventing septic shock comprising a combination of an anti-TNF antibody and IL-6, and a pharmaceutically acceptable carrier.

6. The pharmaceutical composition of claim 5 in which the anti-TNF antibody is an anti-human-TNF antibody and the IL-6 is recombinant human IL-6.

7. The use of a combination of an anti-TNF antibody and IL-6 for the treating or preventing septic shock.

8. The use of a combination of an anti-TNF antibody and IL-6 for the manufacture of a medicament for treating or preventing septic shock.

9. The use of either claim 7 or 8 in which the anti-TNF antibody is an anti-human-TNF antibody and the IL-6 is recombinant human IL-6.

10. A kit comprising in separate containers pharmaceutical compositions for treating or preventing septic shock, one of which containers comprises an anti-TNF antibody in a pharmaceutically acceptable carrier, another of which containers comprises IL-6 in a pharmaceutically acceptable carrier.

11. The kit of claim 10 in which the anti-TNF antibody is an anti-human-TNF antibody and the IL-6 is recombinant human IL-6.

FIG. 1

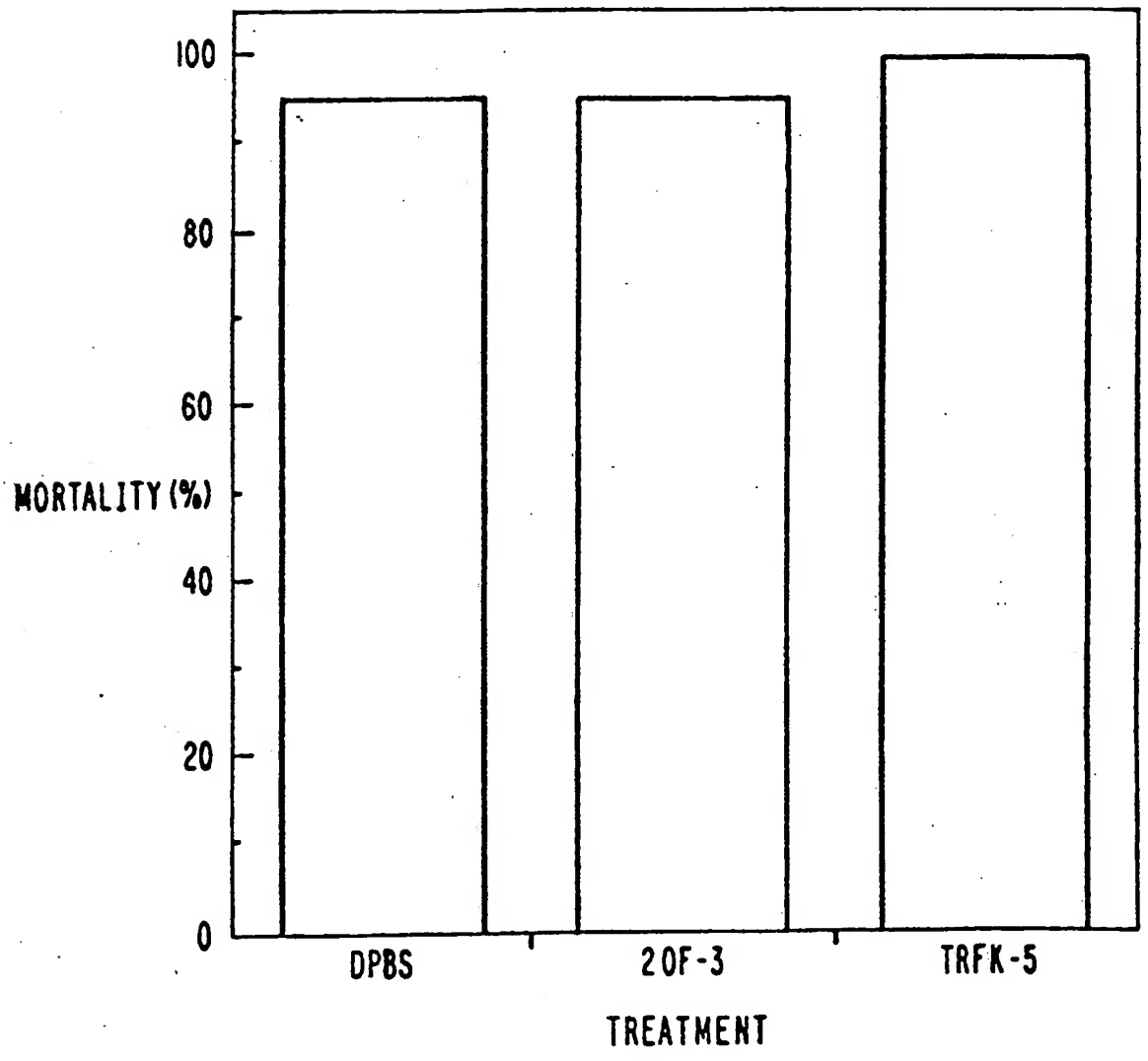


FIG. 2

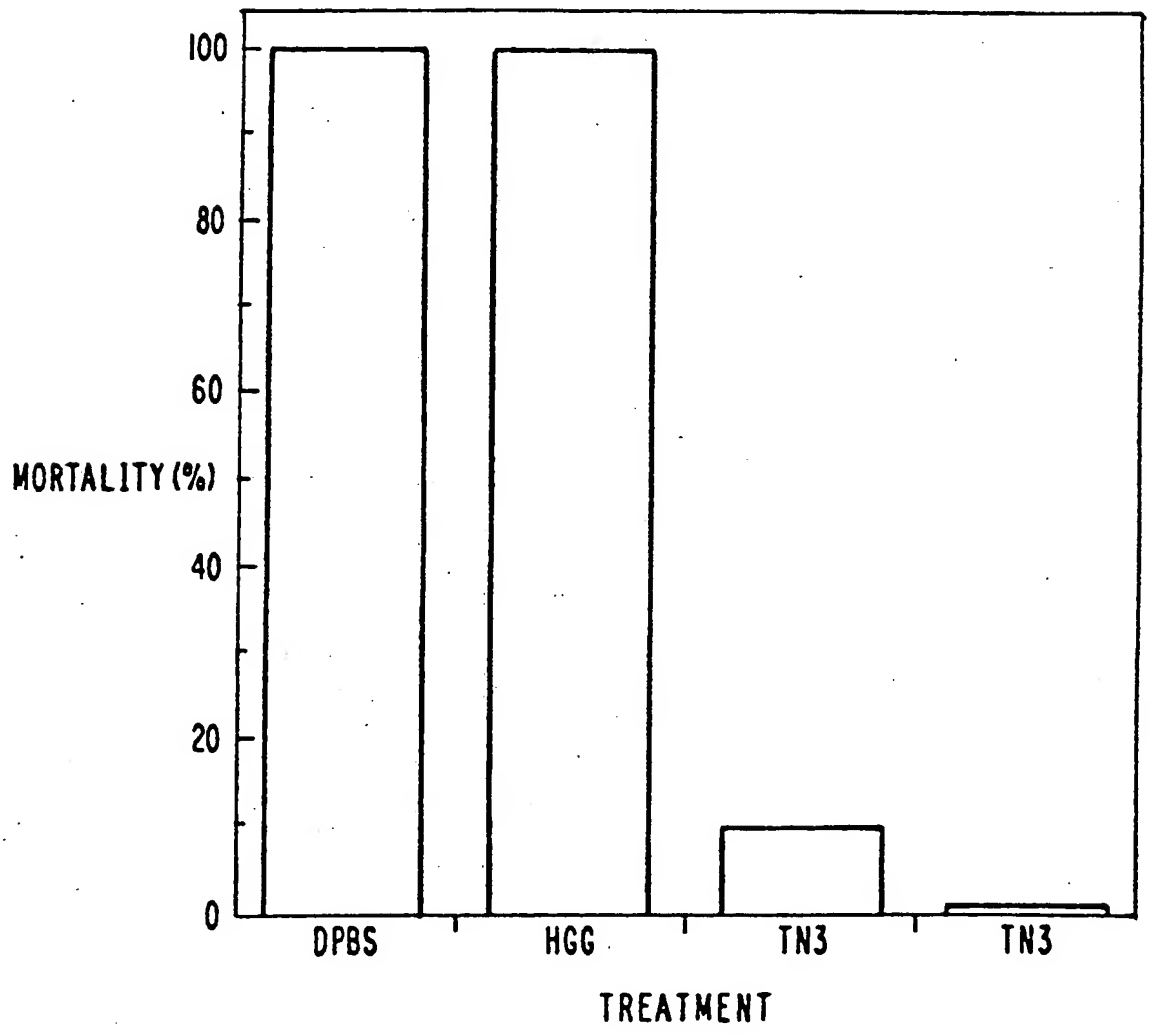
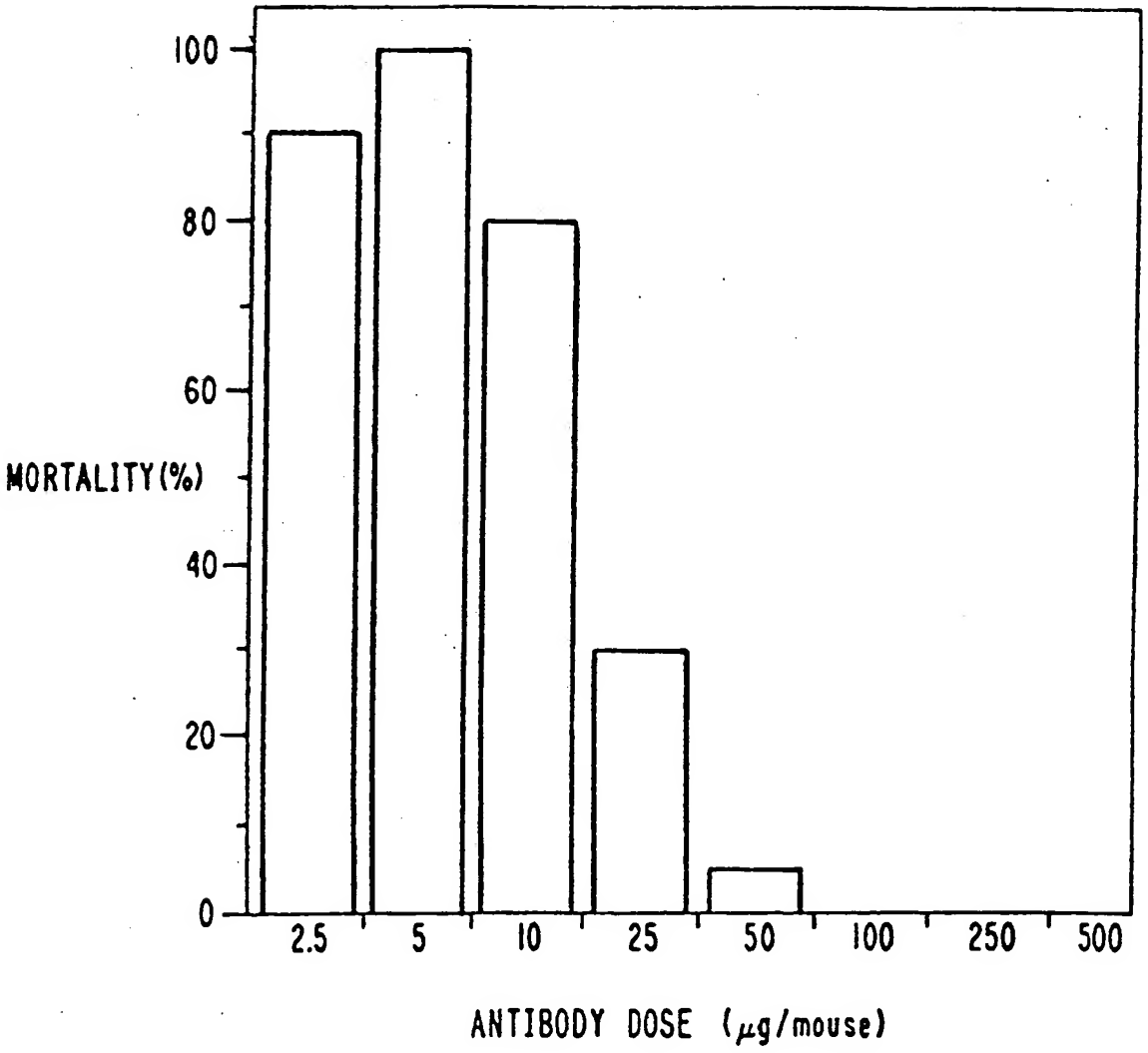


FIG. 3


100
80
60
40
20
0
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5
10
25
50
100
250
500
ANTIBODY DOSE (μg/mouse)

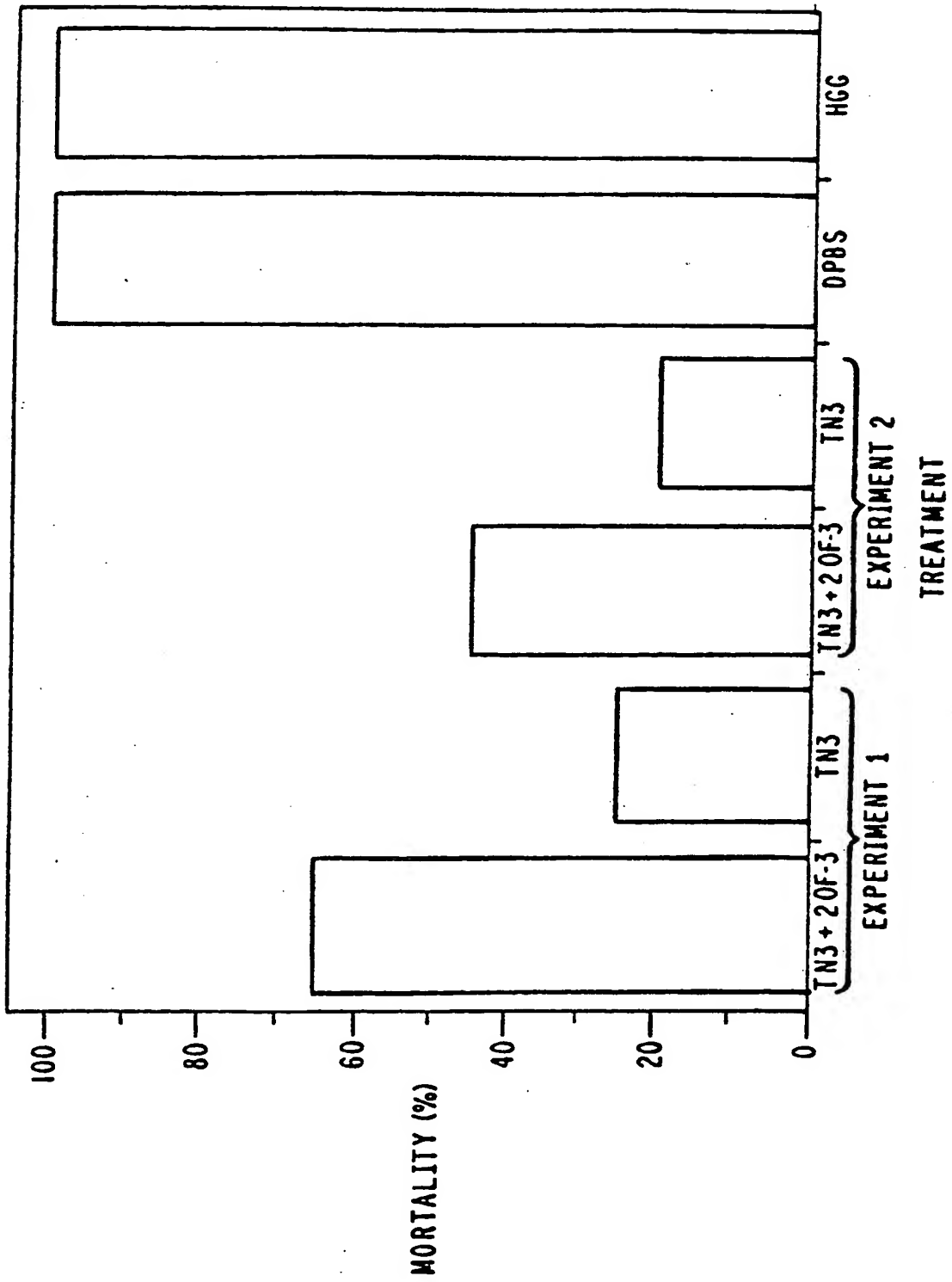

100
80
60
40
20
0
MORTALITY (%)
TN3 + 2OF-3
TN3
TN3 + 2OF-3
TN3
DPBS
HCG
EXPERIMENT 1
EXPERIMENT 2
TREATMENT

FIG. 4

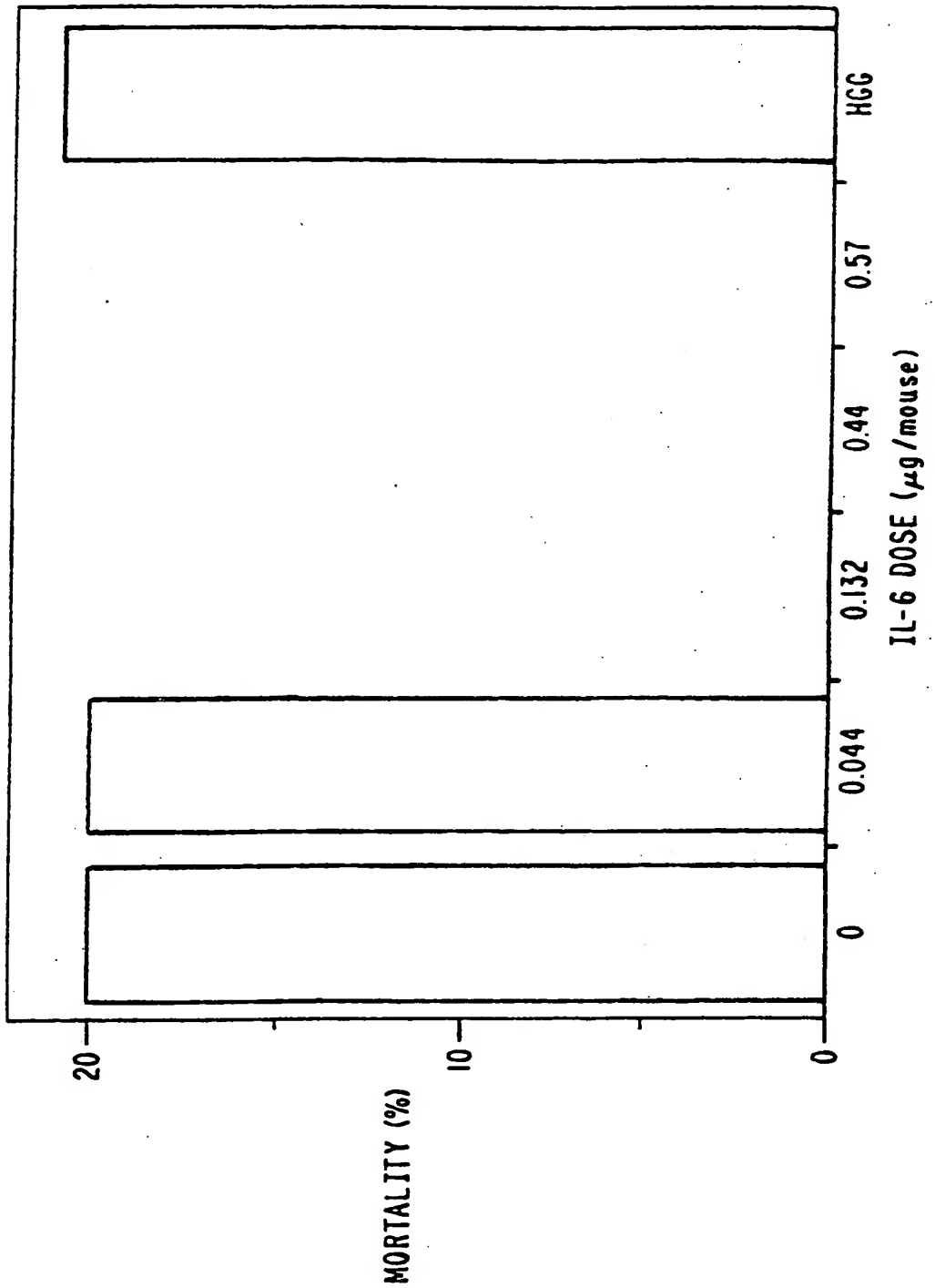
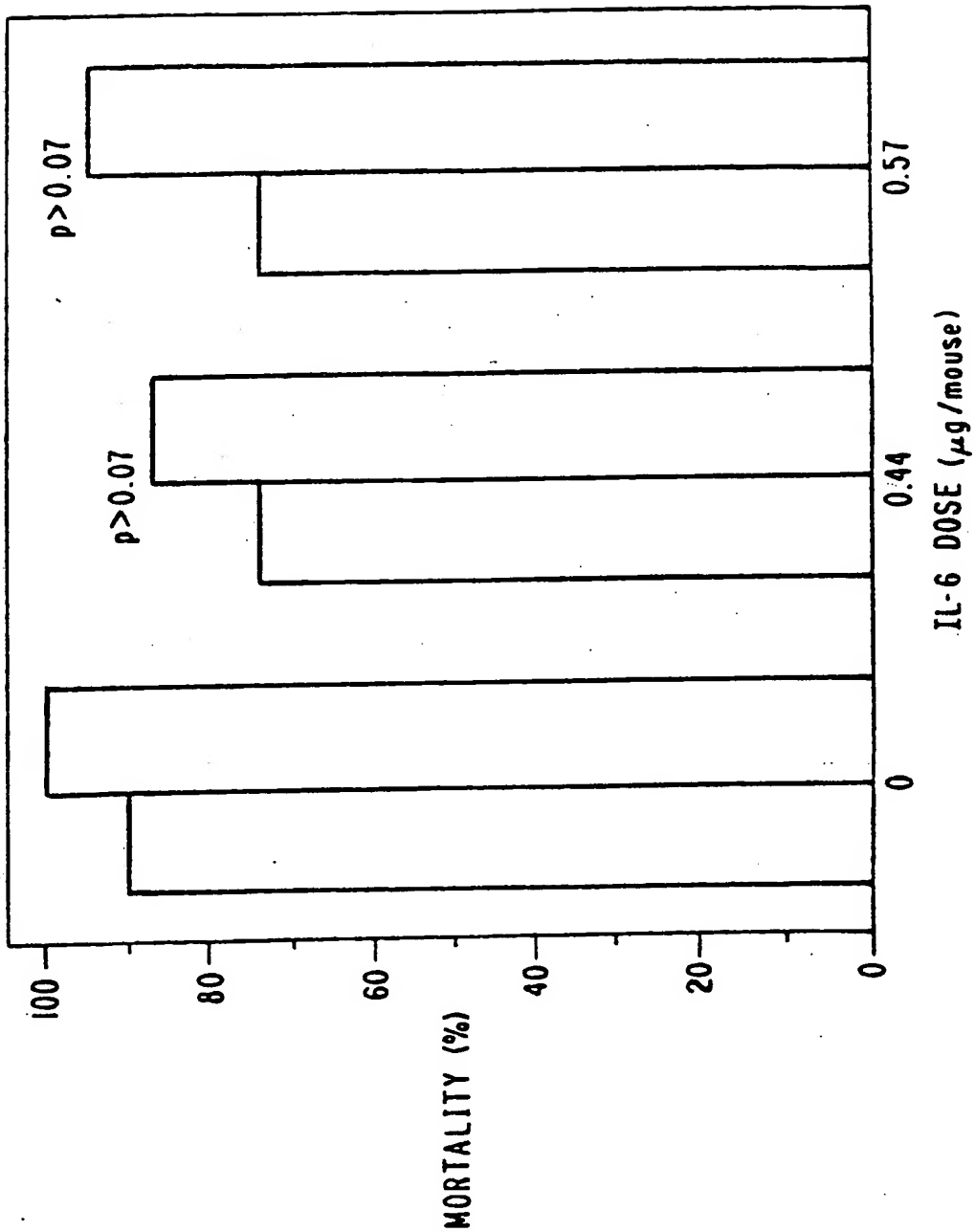


FIG. 5

FIG. 6


p>0.07
p>0.07
100
80
60
40
20
0
MORTALITY (%)
0
0.44
0.57
IL-6 DOSE (μg/mouse)

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K39/395; //(A61K39/395,37:02)

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K ; C07K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
Y	THE JOURNAL OF IMMUNOLOGY vol. 143, no. 11, 1 December 1989, BALTIMORE MD, US pages 3517 - 3523 D. ADERKA ET AL. 'IL-6 inhibits lipopolysaccharide-induced tumor necrosis factor production in cultured human monocytes, U937 cells, and in mice.' cited in the application see abstract; figure 7 see page 3521, right column, line 13 - line 25 see page 3522, left column, line 45 - line 64	1-11
Y	EP,A,0 374 510 (AMERICAN CYANAMID COMPANY) 27 June 1990 see claims	1-11

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12 FEBRUARY 1993	09.03.93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	NOOIJ F.J.M.

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1,2 and 7 (all completely) and 4 and 9 (all partially) are directed to a method of treatment of the animal/human body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9210596
SA 68060

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 12/02/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0374510	27-06-90	AU-B- 624457	11-06-92
		AU-A- 4688589	21-06-90
		CA-A- 2005670	19-06-90
		JP-A- 2212435	23-08-90
		US-A- 5124147	23-06-92